Establishment and Evaluation of a Mouse Model of Allergic Rhinitis

Xiang QIN[1], Yuan PAN[1], Xiaoni PAN[1], Yanfeng MO[2], Shuwen LI[1], Tingting XIE[1], Lixiang LU[1*], Jiancheng LIANG[2*], Shiyou ZHOU[1*], Suoyi HUANG[3,4*]

1. School of Clinical Medicine, Youjiang Medical University for Nationalities, Baise 533000, China; 2. School of Public Health and Management, Youjiang Medical University for Nationalities, Baise 533000, China; 3. School of Pharmacy, Youjiang Medical University for Nationalities, Baise 533000, China; 4. Key Laboratory of Research on Characteristic Ethnic Medicine in the Youjiang River Basin of Guangxi Colleges and Universities, Baise 533000, China

Abstract [**Objectives**] To explore a new method for induction of allergic rhinitis in mice, and compare and evaluate it with common modeling methods. [**Methods**] 36 mice were randomly divided into the control group, blank group and experimental group, and there were 12 mice in each group. The mice in the control group were conventionally induced. That is, the mice were first injected intraperitoneally with the mixture composed of OVA 50 μg, [$Al(OH)_3$] 5 mg and 1ml of normal saline once every other day, and then since the 15th d, 20 μL of 5% OVA solution was dropped into each nasal cavity once a day, which lasted for 7 d. The blank group was treated with the same amount of normal saline according to the control group, and received intraperitoneal injection and bilateral nasal drip respectively. In the experimental group, mice were first given intraperitoneal injection of the mixture composed of ovalbumin (OVA) 75 μg, aluminum hydroxide gel [$Al(OH)_3$] 8 mg and normal saline 1.5 mL for basic sensitization. On the 26th d, 20 μL of 3% OVA solution was dropped into each nasal cavity once a day, which lasted for 10 d. The number of sneezes, the number of nose scratching, the amount of nasal discharge, and the activity of mice in each group were observed, and the behavior of allergic reaction was scored. Meanwhile, the number of eosinophils in the nasal discharge of mice and the IgE content in serum were measured. [**Results**] The score of nasal stimulation symptoms, the number of eosinophils and serum IgE level of mice in the control group and the experimental group were higher than those in the blank group ($P<0.05$), and there was no statistical significance between the two groups in the three indicators ($P>0.05$). [**Conclusions**] The modeling method was more suitable for the development of allergic rhinitis patients' condition, and reduced the probability of death of mice due to modeling, and simplified the experimental operation.

Key words Allergic rhinitis, Mouse model, Modeling effect

1 Introduction

At present, there are many methods of modeling allergic rhinitis in animal experiments, and these methods have their own advantages and disadvantages. For example, Chen Jie *et al.*[1] took mice to be sensitized by intraderitoneal injection of 0.1 mL antigen adjuvant suspension on the 1st and 15th d, and 50 μL of 2 mg/mL OVA was dropped into nasal cavity once a day from the 15th d of the initial sensitization, which lasted for 15 d. The number of sneezing and nose scratching was observed every 3 d for 3 min/mouse, and the modeling time was 30 d. This method has the problem that the initial sensitization interval was too long. Song Chenglin *et al.*[2] injected 0.1 and 0.2 mL of antigen adjuvant suspension containing OVA (1 mg/mL), Bordetella pertussis (1×10^{10}) and $Al(OH)_3$ (2 mg) into the left and right forelimbs and hind limbs of rats for sensitization, and enhanced sensitization 5 d after the initial sensitization. 0.5 mg of OVA dissolving in 1 mL of isotonic saline was injected into the back of rats, and on the 7th d of the initial sensitization, 10 μL of 1 mg/mL OVA was dropped into nasal cavity once a day. This method has the problem of complicated operation and easy to cause accidental death of mice. The commonly used method: basic sensitization was performed 14 d before modeling, and mice were injected with 50 μg OVA + 5 mg $Al(OH)_3$ + 1 mL normal saline mixture, once every other day. On the 15th d, 20 μL of 5% OVA solution was dropped into each nasal cavity once a day, lasting for 7 d[3]. The method has the defect of low drug concentration leading to poor molding effect. In this study, a new modeling method was created through improvement to make up for the shortcomings of the above modeling methods, so as to provide a scientific basis for subsequent research on the mechanism of allergic rhinitis and the development of treatment methods.

2 Materials and methods

2.1 Materials

2.1.1 Animals. 36 ICR-grade mice (12 - 15 g) aged 3 - 4 weeks (half male and half female) were purchased from Changsha Tianqin Biotechnology Co., Ltd., and the production license number is SCXK(Xiang)2022-0011. They were raised in the Animal Laboratory Center of Youjiang Medical University For Nationalities, where indoor ventilation was good, and indoor temperature was 19 - 22 ℃. The feed conformed to the standard experimental mouse feed. Sterile pure water was replaced once every two days, and bedding was replaced 3 times a week.

2.1.2 Drugs and reagents. Ovalbumin (OVA), 4% aluminum hydroxide gel [$Al(OH)_3$], Reye dye, and mouse immunoglobulin E (IgE) ELISA kit were purchased from Nanning Baishi Innovation Biotechnology Co., Ltd. in Guangxi.

2.1.3 Main instruments. Main instruments included multifunctional enzyme marker (Mithras LB 943, Berthold, Germany), table high-speed centrifuge (TG16-WS, Hunan Xiangyi Laboratory Instrument Development Co., Ltd.), intelligent constant temper-

Received: March 9, 2024 Accepted: July 15, 2024
Supported by the National Innovation and Entrepreneurship Project for University Students (202110599012).
* Corresponding author. E-mail: 190023154@qq.com; 2961102720@qq.com; 281487859@qq.com; huangsuoyi@163.com

ature water tank (HH-Zk600, Gongyi Yingyu Gaoke Instrument Factory), inverted microscope (Leica DMi8, Germany Leica Company), and electronic balance (FA1204B, Shanghai Tianmei Balance Instrument Co., Ltd.).

2.2 Methods

2.2.1 Preparation of reagents. Reagent 1: OVA 50 μg + [$Al(OH)_3$] 5 mg + 1 mL normal saline mixture. Reagent 2: OVA 75 μg + [$Al(OH)_3$] 8 mg + 1.5 mL normal saline mixture.

2.2.2 Grouping. The animals were randomly divided into the control group, blank group and experimental group, with 12 mice in each group (half male and half female).

2.2.3 Modeling method. Control group: basal sensitization was performed 14 d before modeling, and reagent 1 was injected into mice intraperitoneally, once every other day. On the 15th d, 20 μL of 5% OVA solution was dropped into each nasal cavity once a day for 7 d[3]. Blank group: the same amount of normal saline was used as the control group. Experimental group: on the 1st, 3rd, 10th and 19th d, reagent 2 was injected into mice intraperitoneally for basal sensitization. On the 26th d, 3% OVA solution was dropped into each nasal cavity for 10 d.

2.2.4 Model determination. The nasal symptoms and signs of mice were the main observation indicators. After the severity, frequency and duration of nasal itchiness, sneezing and runny nose were scored 15 min after the self-supporting drug was dropped into each nasal cavity, and the score was recorded[4]. The scoring criteria were shown in Table 1.

Table 1 Behavioral rating table of anaphylaxis

Symptom	Score (15 min after stimulation)		
	1	2	3
Nasal itchiness	≤2	Between the two	Scratching the nose violently
Sneezing	1 –	3 –	11 –
Runny nose	Flowing to the anterior nostril	Flowing beyond the anterior nostril	Tears streaming down one's face

2.2.5 Smear of nasal secretion. The nasal secretion of each group of mice were taken with a small cotton swab for smear. The smears were dried by natural means and dyed with Wright's A stain for 1 min. Afterwards, Wright's B stain phosphate buffer that was 2 – 3 times of Wright's A stain was dropped on the smears, and after being gently shaken, they were left for 5 min. They were washed and sucked dry, and the morphological changes and quantity of eosinophils were observed under a light microscope. Each section was randomly selected with 5 high-power fields of view for counting.

2.2.6 Measurement of serum IgE by double-antibody sandwich ELAISA method. The lgE level was detected according to the ELAISA kit[5].

2.2.7 Statistical methods. SPSS 26 software was used to establish a database and conduct statistical analysis. The data was represented by ($\bar{x} \pm s$), and one-way ANOVA analysis of variance was used. The test level: $\alpha = 0.05$ (both sides), and $P < 0.05$ means the difference was statistically significant.

3 Results and analysis

3.1 General signs of mice All mice survived during the experiment. The mice in the blank group were normally active, and had no runny nose, sneezing and nose scratching. The 24 mice in the control group and the experimental group had symptoms of nasal scratching, runny nose, and sneezing. In the control group, the score of anaphylaxis behavior of 7 mice was above 5. In the experimental group, the score of anaphylaxis behavior of 8 mice was above 5. The score of the control group and the experimental group was higher than that of the blank group ($P < 0.05$), but there was no statistical significance between the two groups ($P > 0.05$) (Table 2).

Table 2 Score of nasal stimulation symptoms in mice

Group	Number of samples	Score of symptoms
Blank	12	0.750 ± 0.754
Control	12	4.750 ± 1.215[a]
Experimental	12	4.580 ± 1.505[a]

NOTE In the analysis of variance $F = 42.763$, $P < 0.001$; [a] means that compared with the blank group, $P < 0.05$.

3.2 Number of eosinophils in nasal secretion The number of eosinophils in the nasal secretion of experimental mice in both the control group and the experimental group were higher than that in the blank group ($P < 0.05$), but there was no statistical significance between the two groups ($P > 0.05$) (Table 3).

Table 3 Number of eosinophils in the nasal secretion of mice

Group	Number of samples	Number of eosinophils
Blank	12	2.33 ± 0.99
Control	12	6.58 ± 1.56[a]
Experimental	12	6.92 ± 1.93[a]

NOTE In the analysis of variance $F = 32.942$, $P < 0.001$; [a] means that compared with the blank group, $P < 0.05$.

3.3 Serum IgEμg level The serum IgEμg level of mice in both the control group and the experimental group were higher than that in the blank group ($P < 0.05$), but there was no statistical significance between the two groups ($P > 0.05$) (Table 4).

Table 4 Detection results of serum IgE level in mice

Group	Number of samples	IgE// μg/mL
Blank	12	0.734 ± 0.140
Control	12	1.795 ± 0.287[a]
Experimental	12	1.963 ± 0.264[a]

NOTE In the analysis of variance $F = 93.004$, $P < 0.001$; [a] means that compared with the blank group, $P < 0.05$.

4 Discussion

Allergic rhinitis is the most common and frequent disease in ear, nose and throat department. According to statistics, the incidence of allergic rhinitis in children and adults is 40% and 10% – 30%, respectively, and its causes include inheritance, air pollution, and bronchial asthma[6]. Its symptoms include itchy nose, stuffy nose, runny nose, and sneezing[7].

The establishment of animal models is an important method to study human diseases[8]. Various factors affecting the development of the disease can be observed. It can also be applied to human beings through the study of the life phenomena of animals themselves. The ideal modeling method for animals must have the characteristics of high success rate, high survival rate and simple operation[9]. At present, there are different methods for establishing allergic rhinitis models at home and abroad, which are mainly achieved through systemic and local sensitization of allergens. Rodents are mainly selected, including rats, mice, guinea pigs, *etc.* Although rats have a high success rate in modeling, their large size increases the difficulty and cost of experimental operation. There are a large number of complement in the serum of guinea pigs, and it is a biological feature that is easy to sensitize, but the modeling effect is poor and the success rate is low. Therefore, mice were selected in this experiment, and they were easy to operate and had certain biological characteristics of sensitization. The allergens used for modeling mainly include ragweed pollen, TDI, OVA, fine worms and fungi[10], among which TDI and OVA are commonly used. Studies have shown that TDI has certain toxicity, and if it is used in animal modeling, animal mortality is higher; it also has certain hazards to experimental personnel, and lacks stability and safety[11]. OVA is in powder form and has strong immunogenicity, but it is easy to coagulate and denature in a solution, so aluminum hydroxide gel is often used as adjuvant. It is found that the effect of aluminum hydroxide gel on enhancing the allergic reaction model caused by ovalmin is better than that of aluminum hydroxide dry powder model[12]. Hence, in this study, OVA was used as the allergen, and aluminum hydroxide gel was as the adjuvant to successfully build an animal model of allergic rhinitis; no adverse phenomena such as granuloma were found in the organs of mice[13]. In recent years, experimental studies have also shown that willow pollen protein extract, as an allergen, can also be used for successful molding[14]. The establishment of mouse model of allergic rhinitis consists of two stages, basic sensitization and local excitation. Common methods include aerosol inhalation, nasal inhalation, and intraperitoneal injection, of which aerosol inhalation will cause sensitization of lower respiratory tract. In most studies, intrabitoneal injection was used for basic sensitization, and nasal inhalation was used for local stimulation. The method is mature and has high stability and low experimental cost, so the method of intrabitoneal injection and nasal inhalation was used for modeling in this experiment[15]. During the establishment of the model in this study, the control group was treated with the commonly used modeling method in previous studies, namely reagent 1: OVA 50 μg + [$Al(OH)_3$]5 mg + 1 mL normal saline mixture. The administration method was as follows: basic sensitization was performed 14 d before modeling, and the mice were injected with reagent 1 normal saline mixture peritoneally once every other day. On the 15th d, 20 μL of 5% OVA solution was dropped into each nasal cavity once a day for 7 d. The experimental group adopted the improved method, namely reagent 2: OVA 75 μg + [$Al(OH)_3$] 8 mg + 1.5 mL normal saline mixture. The administration method was as follows: on the 1st, 3rd, 10th and 19th d, the mice were injected with reagent 2 normal saline mixture for basic sensitization. On the 26th d after basic sensitization, 3% OVA solution was dropped into bilateral nasal cavities for 10 d. There was no accidental death or asthma of mice during the experiment.

The modeling scoring criteria reported in previous studies are basically consistent[4]. The behavior indicators of mice after modeling were scored, including the number of sneezes, the degree of runny nose, and the degree of scratching nose and face. The total score greater than 5 means the success of modeling. A large number of studies have shown that patients with allergic rhinitis contain a large number of inflammatory cells, in which eosinophils and their released toxic proteins play an important role in the course of disease development[16]. Allergic rhinitis belongs to type I allergy, and secretory immunoglobulin IgE is an important antibody to induce type I allergy, which is mostly derived from lamina propria cells of nasopharynx, gastrointestinal mucosa, and bronchus[17]. In this study, it was found that there was no statistical significance between the two groups in the score, the number of eosinophils and serum IgE level of mice in the control group and the experimental group ($P > 0.05$). Meanwhile, the number of eosinophils and serum IgE level of mice in the control group and the experimental group were higher than those in the blank group ($P < 0.05$).

In summary, on the basis of the original sensitization method, according to the characteristics of long course and continuous stimulation of allergic rhinitis, this experiment appropriately extended the modeling time, increased the frequency of sensitization in the basic sensitization stage, increased the drug concentration within the acceptable safe range of mice, ensured the success rate of modeling, and reduced the accidental mortality of mice, and had a good modeling effect.

References

[1] CHEN J, YANG T, LAI XS, *et al.* Effect of acupoints external application of herbal drug on blood cell classification and serum specific IgE level of allergic rhinitis mice[J]. Journal of Guangzhou University of Traditional Chinese Medicine, 2008, 25(4): 304 – 306. (in Chinese).

[2] SONG CL, ZHANG ZX, CHENG ZD, *et al.* "The Three Needles" acupuncture to allergic rhinitis rat model IL-4, and behavior of the experimental observation[J]. Chinese Archives of Traditional Chinese Medicine, 2011, 29(9): 2139 – 2141. (in Chinese).

[3] TIAN Y, ZHANG F, DUAN CX. The effect of astragaloside Ⅳ on HMGB1/TLR4/NF-κB signaling pathway in mice with allergic rhinitis[J]. Current Immunology, 2021, 41(1): 50 – 55. (in Chinese).

[4] YAN WM, LONG P, YANG JS, *et al.* Research on the alleviating effect of hydrogen gas on allergic rhinitis in mice[J]. Laboratory Animal Science, 2021, 38(2): 35 – 41. (in Chinese).

[5] CHENG NS. An investigation on the effects of dexamethasone on TGF-B level in the serum and nasal septal mucosa of mouse with experimental allergic rhinitis[D]. Taiyuan: Shanxi Medical University, 2010. (in Chinese).

(To page 121)

gout: The epidemiological trends, pathophysiology, clinical presentation, diagnosis and treatment[J]. Journal of the Pakistan Medical Association, 2021, 71(4): 1234 – 1238.
[7] GUO H, TING JP. Inflammasome assays in vitro and in mouse models [J]. Current Protocols in Immunology, 2020, 131(1): e107.
[8] LIU YR, WANG JQ, LI J. Role of NLRP3 in the pathogenesis and treatment of gout arthritis [J]. Frontiers in Immunology, 2023 (14): 1137822.
[9] SO AK, MARTINON F. Inflammation in gout: Mechanisms and therapeutic targets[J]. Nature Reviews Rheumatology, 2017, 13(11): 639 – 647.
[10] MADHOGARIA B, BHOWMIK P, KUNDUA. Correlation between human gut microbiome and diseases[J]. Infections in Medicine, 2022, 1 (3): 180 – 191.
[11] SONG S, LOU Y, MAO Y, *et al.* Alteration of gut microbiome and correlated amino acid metabolism contribute to hyperuricemia and Th17-Driven inflammation in Uox-KO mice [J]. Frontiers in Immunology, 2022(13): 804306.
[12] DALBETH N, GOSLING AL, GAFFO A, *et al.* Gout[J]. Lancet, 2021, 397(10287): 1843 – 1855.
[13] SUNKUREDDI P. Gouty arthritis: Understanding the disease state and management options in primary care[J]. Advances in Therapy, 2011, 28(9): 748 – 760.
[14] SCHLESINGER N. The safety of treatment options available for gout [J]. Expert Opinion on Drug Safety, 2017, 16(4): 429 – 436.
[15] ZHU J, ZHENG Z, LIU Y, *et al.* The effects of small-needle-knife therapy on pain and mobility from knee osteoarthritis: A pilot randomized-controlled study [J]. Clinical Rehabilitation, 2020, 34 (12): 1497 – 1505.
[16] ZHAO Y, FANG W, QINKAI Z. Mechanistic considerations in the closed treatment of myofasciitis with the small-needle knife[J]. Chinese Acupuncture and Moxibustion, 2014, 34(9): 907 – 909. (in Chinese).
[17] LIUCAN K, XIANGDON D, QIAOJIN L. Therapeutic effect of needle knife combined with ozone application on osteoarthritis of the knee joint observed[J]. Journal of Guiyang College of Traditional Chinese Medicine, 2014, 36(2): 143 – 146. (in Chinese).
[18] LINWEI X, LIU B. Efficacy and mechanism of action of ultraminimally invasive needle knife microscopic plasioplasty in the treatment of early osteoarthritis of the knee[J]. Journal of Guizhou Medical University, 2024, 49(3): 430 – 435,442. (in Chinese).
[19] DINGXIAO J, CHENMIN Z, ZHANGYI J, *et al.* Progress in the clinical application of minimally invasive needlestick microscopy for the treatment of joint-like diseases[J]. Rheumatism and Arthritis, 2023, 12 (4): 67 – 70. (in Chinese).
[20] WUJI J, CHENGMEI F, YUNLAN B. Expert consensus on the prevention and treatment of gout in Dai medicine[J]. Chinese Journal of Ethnomedicine and Ethnopharmacy, 2022, 31(8): 1 – 4. (in Chinese).
[21] HUANG Y, YUNLAN B, YUN H, *et al.* Dai medicine treatment programme for Longshahou (gout) [J]. Chinese Journal of Ethnic Medicine, 2015, 21(1): 23 – 24. (in Chinese).
[22] PANLI B, WANGXIAO M, HUANG Y. Summary of the experience of Dai medicine practitioners in diagnosing and treating acute gouty arthritis [J]. Guiding Journal of Traditional Chinese Medicine and Pharmacy, 2017, 23(5): 37 – 40. (in Chinese).
[23] XIAOYON H, WANGXIN H, PENGJIAN Y, *et al.* Characteristics of the four major ethnomedicine treatments for gout disease[J]. Chinese Journal of Ethnomedicine and Ethnopharmacy, 2017, 26(2): 62 – 64. (in Chinese).
[24] FEIFAN L, FANYI S, YUANMEI B, *et al.* Mechanism of DaiTongXiao in the treatment of gouty arthritis through the NLRP3 signaling pathway [J]. Journal of Ethnopharmacology, 2023, 319(P3): 117313.

(From page 113)

[6] HONG YG. The etiology, treatment status and influencing factors of anaphylactic rhinitis[J]. China Medical Innovation, 2018, 15(11): 144 – 148. (in Chinese).
[7] WANG MY, WANG W, WANG SM, *et al.* Effect of intraperitoneal injection of ovalbumin on mouse respiratory sensitization model[J]. Journal of Youjiang Medical University for Nationalities, 2023, 45(3): 439 – 444. (in Chinese).
[8] MCGONIGLE P, RUGGERI B. Animal models of human disease: Challenges in enabling translation[J]. Biochemical Pharmacology, 2014, 87 (1): 162 – 171.
[9] DONG PL, YIN X, ZHANG TY, *et al.* Comparison of Allergic Rhinitic Models Induced by Various Allergens[J]. China Pharmacist, 2014, 17 (2): 196 – 198. (in Chinese).
[10] ASARIA M, DHAMI S, VAN RR, *et al.* Health economic analysis of allergen immunotherapy for the management of allergic rhinitis, asthma, food allergy and venom allergy: A systematic overview[J]. Allergy, 2018, 73(2): 269 – 283.
[11] KANEYASU M, NAGATA M, IKEDA H, *et al.* Anti-allergic activity of lotus root (*Nelumbo nucifera*) powder in TDI-sensitized nasal allergy model mice[J]. Food and Agricultural Immunology, 2019, 30(1): 968 – 978.
[12] DONG F, TANG HW, WU XL, *et al.* Efficiency of rat asthma model established with aluminum hydroxide powder and gel[J]. Academic Journal of Chinese PLA Medical School, 2013, 34(1): 73 – 75. (in Chinese).
[13] LI J, LI Y. Autophagy is involved in allergic rhinitis by inducing airway remodeling[J]. International Forum of Allergy & Rhinology, 2019, 9 (11): 1346 – 1351.
[14] LUO ZL, XUAN R, XIN LL, *et al.* Specific desensitization therapy forallergic rhinitis caused by willow pollen[J]. Laboratory Animal Science, 2020, 37(4): 10 – 14. (in Chinese).
[15] HU YF, TANG F. Establishment of animal models of allergic rhinitis: An update[J]. Journal of Medical Postgraduates, 2012, 25(5): 540 – 543. (in Chinese).
[16] LIN CP, LEI XY, TANG J, *et al.* The value of eosinophil level in nasal secretions of patients with allergic rhinitis in assessing the severity of the disease[J]. The Journal of Medical Theory and Practice, 2022, 35 (13): 2288 – 2289, 2307. (in Chinese).
[17] ZHANG JJ. The Expression and clinical significance of IgE, IFN-γand IL-8 in serum of patients with allergic rhinitis[J]. Labeled Immunoassays and Clinical Medicine, 2022, 29(9): 1497 – 1500. (in Chinese).